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On the Cytoplasmic Constituents of Uroleptus Mobilis, Engelmann

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DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

By

FLORENCE DE L. LOWTHER

NEW YORK, N. Y.

EASTON, PA.
MACK PRINTING COMPANY
DEC., 1925

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ON THE CYTOPLASMIC CONSTITUENTS OF UROLEPTUS MOBILIS (ENGELMANN)

FLORENCE DE LOISELLE LOWTHER

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(without figures)

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INTRODUCTION

In recent years attention has been focused on the morphological and physiological study of the extra-nuclear elements of the metazoan cell, such as mitochondria, Golgi apparatus and various types of granules. The extra-nuclear components of Protozoa, however, apart from kinetic elements, have not been examined with the same thoroughness and until recently have received little attention. Since in any given protozoan all the vital activities are taking place in the one cell, the further study of its cytoplasmic elements might well prove of interest; though it must be admitted that the unraveling of the functions of any one of them, because of the complexity of activities within so small a compass, becomes correspondingly difficult.

The presence of mitochondria in Protozoa has been described by a number of investigators, chief among whom is Fauré-Fremiet ('08, '09, '10, '12). Other contributors to our knowledge of mitochondria are Benda ('99), Hirschler ('14, '24), Arndt ('14), Alexeieff ('16, '17), Leger and Duboscq ('16), Dehorne ('21), Cutler ('22), Joyet-Lavergne ('24a, b, c) and Nassanov ('24).

A Golgi apparatus was first described in Protozoa by Hirschler ('14, '24) in connection with Sporozoa, and has since been found in the same group by King and Gatenby ('23) and Joyet-Lavergne ('24a, b, c); while Nassanov ('24) has produced evidence that the contractile vacuole of Protozoa has a permanent membrane which he considers the possible homologue of the Golgi apparatus of the metazoan cell.

Different types of granules have been noted. Among these may be listed the "Exkretperlen" of Wallengren ('97) and Prowazek ('98) and Slonimski and Zweibaum ('22) which may appear on the periphery of some forms. The "Exkretkörner" of Schewiakoff which Maupas ('84) called "Corpuscules Biréfringents" may also be included here.

In the following study an effort has been made to determine elements which may appear in the cytoplasm of Uroleptus mobilis and to trace their history through the various phases of its life cycle,

determining, if possible, any interrelationships between these elements and the nucleus.

MATERIAL

Prof. Gary N. Calkins suggested the use of *Uroleptus mobilis*, Engelmann, an hypotrichous ciliate which he has had under cultivation since 1917, as a convenient form upon which to work. This animal can be cultivated in large masses and every phase of the life cycle may be obtained without difficulty.

Morphology

The animal under observation was found by Calkins in an old culture in the laboratory of Columbia University. The type varies somewhat from that described for the first time by Engelmann, which led Calkins to call it the "New York" variety. It possesses five rows of cilia on the ventral and ventro-lateral surfaces and has but three cirri situated at the ventral surface of the anterior end. A well-developed adoral zone of membranelles is present on the right side which almost fills the region of the peristome, while two membranes, endoral and paroral, can be made out. The cilia are long and rather sparsely placed. In the anterior region they are unusually long and there is some evidence that their general distribution is in groups of two. A contractile vacuole is located in the center of the body near the dorsal surface. The protoplasm is dense and alveolar making it difficult to determine structures in life. There are usually from eight to ten macronuclei while the micronuclei may vary in number from two to six. The average length as computed by Calkins of animals fed on a hay-flour medium which has been diluted by spring water is 140 to 165 μ and the diameter 11 to 17 μ. Animals fed on a richer diet may grow a third again as large. The cysts are spherical and the cyst wall smooth with an average diameter of 32.7 μ.

Life Cycle

The life history of the organism was described by Calkins ('19) as follows: *Uroleptus mobilis* divides repeatedly for a number of weeks and then, under proper conditions, may either conjugate or encyst. The result of conjugation or encystment

gives rise to a rejuvenescence or renewed vitality of the protoplasm, as measured by the increased division rate and increased longevity, while failure to conjugate or to encyst eventually brings about senescence and death. The nuclear changes taking place throughout the active phases of the life cycle have been described by Calkins with the exception of the possible changes during encystment. S. C. Dellinger is now working out the finer nuclear reorganization in this phase of the life cycle.

TECHNIQUE

1. The living organism.

(*A*) *Culture Methods*

The food medium used by Calkins in his study of *Uroleptus* is as follows:

100 mg. of chopped timothy hay
130 mg. of flour (which has not been bleached with chlorine)
100 cc. of spring water

This is boiled for ten minutes or more and allowed to stand for twenty-four hours after which at least an equal amount of fresh spring water is added. I find, however, that animals become larger and contain conspicuous food vacuoles if given the medium without dilution.

Mass cultures of *Uroleptus* were grown in Syracuse dishes and transferred from time to time to new dishes filled with fresh medium. To obtain veritable epidemics of division it is only necessary to transfer a few pipettes full of the animals to a fresh medium and in thirty-six hours organisms in every stage of division may be had in great abundance. Epidemics of conjugation and encystment are frequent in mature cultures in which the medium has not been changed for some time. It is therefore a simple matter to obtain material for study in any and all stages of the life cycle of this form.

(*B*) *Intra-vitam Methods*

1. Neutral red was employed for the following purposes:

(*a*) To determine the presence of granules and to trace them through the various stages of the life cycle *in vivo*.

A minute drop from a drawn out pipette of a .1% solution of neutral red added to 5 cc. of culture medium is sufficient to color certain granules of the animals contained in that entire culture. Differences in intensity of color due to temperature changes or concentration of animals as determined by Slonimski and Zweibaum ('22) were not considered. The addition of neutral red does not seem to injure the *Uroleptus* as they continue to divide, conjugate and encyst. This is substantiated by Prowazek ('98) in his study of "Exkretperlen" of *Paramecium* where he finds the division continues after the application of neutral red.

(*b*) To determine the presence of food vacuoles and their contents.

(*c*) To aid in preserving certain granules which when treated with alcoholic fixatives would otherwise be dissolved. See infra under Schaudinn fixative.

2. Janus Green-B. An approximate concentration of 1:10,000 was used as a specific indicator for the presence of mitochondria as advocated by Fauré-Fremiet for Protozoa and by Ehrlich, Michaelis, Laguesse, Cowdry, Gatenby, M. R. Lewis, and a host of others for Metazoa.

A minute drop of Janus Green was applied to a slide and into this was placed another drop of medium of equal size containing a rich culture of *Uroleptus* and a cover slip applied. Mitochondria could be detected from fifteen to thirty minutes after application of Janus Green. When the medium on the slide dries down the animals tend to disintegrate at which time mitochondria can be made out with additional clarity as they pour out from the ruptured cortex of the cell along with the rest of the cytoplasm into the medium.

(*C*) *Culture Methods for Bacteria*

Equal parts of agar and hay-flour infusion were mixed and placed in petri dishes and test tubes under the proper sterile conditions.

Smears of ordinary medium containing bacteria were placed upon the agar-medium plates and when cultures of different bacteria were evident pure cultures were isolated from these and placed in test tubes.

Smears of washed animals were made as follows: five *Uroleptus* were isolated under a binocular and each animal was washed a number of times in a succession of drops of sterile medium, a new sterile pipette and drop being used for each operation. Each washed animal was then smeared upon a marked-off strip of agar-medium plate and put aside to await development of any possible bacteria.

(*D*) *Polarized Light*

Polarized light has been used by Schewiakoff and Maupas to determine the presence of granules of an excretory nature composed of calcium phosphate to be found in vacuoles and cytoplasm after feeding with meat extracts and hay infusion and other organisms. These "Exkretkörner" under crosssed nicols of the polariscope shine as bright spots of light. This method was applied to *Uroleptus* with excellent results.

Polarized light has also been employed by Gatenby to reveal the presence of mitochondria in fresh material. The mitochondria of *Uroleptus*, however, displayed no evidence of birefringence.

2. Methods of Fixation.

(*A*) *Fixation of Totals*

1. When using an alcoholic fixative, such as Schaudinn fluid, a minute drop of medium containing a large number of animals is placed on an albumin smeared cover slip and the fixative applied. As soon as the alcohol dries the cover slip is plunged into water. The total process should not take more than a minute.

2. When a non-alcoholic fixative is employed a large number of animals in a minimum amount of medium are placed in shallow salt cellars such as Calkins uses for isolation cultures. The fixative is then applied and the dish covered over for the requisite length of time. Test tubes with ground glass stoppers were used when prolonged osmic treatment was called for. The animals are then transferred through a succession of dishes under a binocular, for washing and dehydration. When they have been dehydrated through 95% alcohol they are removed

to cover slips smeared with albumin and the usual process repeated. Although this seems to be an arduous method it is a surer one than that of centrifuging after each operation.

(*B*) *Fixation for Sections*

Large numbers of animals along with zooglea in a minimum quantity of medium are placed in shallow dishes and the same method employed as for killing with non-alcoholic fixatives. When 95% is reached eosin is added to give color to the mass. For embedding the dishes are kept warm over an electric lamp and transfers are made under a binocular with a capillary pipette to liquid paraffin. Sections should never be more than 4 microns thick.

(*C*) *Fixatives Used*

1. For Nuclei and General

Schaudinn fixative made up of 100% alcohol and corrosive sublimate to saturation point is not only a most satisfactory fixative for nuclei but has also proved effective for mitochondria if used for only a fraction of a minute. This can be explained when it is made clear that there is a minimum amount of water present making it possible for the alcohol to evaporate almost instantaneously. The cover slip is then placed in water and the alcohol has not had time materially to alter mitochondria.

Some lipoid structures which would normally be dissolved by Schaudinn fluid can be preserved if neutral red is added before fixation. This seems to render them insoluble though it is not always completely effective.

2. Fixatives for Mitochondria

(*a*) Benda, Bensley and Meves fixatives were all found to be effective.

(*b*) Two per cent osmic acid used for a period ranging from one to thirty-six hours followed by turpentine preserves the mitochondria but does not blacken them. As Fauré-Fremiet also found, acid Fuchsin, Orange G or Licht Grün must be used in connection with this fixative.

(*c*) Equal parts of 2% osmic acid and 5% potassium bi-

chromate for a period of twenty-four hours or more preserves both mitochondria and Golgi elements though neither are much blackened. This fixative is often superior to the 2% osmic acid.

(*d*) Schaudinn fixative (see above)

3. Golgi Fixatives

(*a*) Mann's sublimate-osmic as modified by Bowen (*i. e.*, equal parts of saturate solution corrosive sublimate in physiological saline and 2% osmic) for a day, followed by 2% osmic for several days is the most effective fixative. This tends to blacken the rodlets as no other fixative seems to do.

(*b*) Nassanov's technique (equal parts of 6% potassium bichromate, 1% chromic acid, 2% osmic acid, followed by post osmication for from two to four days) has been found effective.

(*c*) Two per cent osmic acid for a period ranging from one to three weeks has also proved successful.

4. Peripheral Granules

Peripheral granules are all well fixed by the 2% osmic acid, osmic and potassium bichromate combinations, the Kopsch and the Giemsa methods.

(*D*) *Staining Methods*

The stains used were Benda's crystal violet, and Bensley's and Meve's stains. Haedenhain's iron haematoxylin was also used extensively and counterstains of acid Fuchsin, Licht Grün, Orange G or Eosin.

(*E*) *Bacterial Technique*

Two stains were used to determine the presence of bacteria in the animals and in the medium.

1. Gram's method, as follows: Several animals were permitted to dry on a slide. Gram's Sterling formula was added followed by washing in tap water. Gram iodine was then applied and washed off in 95% alcohol until all color was drained off. Fuchsin or Safranin was then used as a counterstain, and the slide allowed to dry. This method colors bacteria in the medium and in the food vacuoles.

2. Giemsa stain: To a concentrated mass of dried animals on a slide was added Grubler's Giemsa with four times quantity of distilled water, washed and differentiated in 95% alcohol followed by 100% alcohol, toluol, and mounted in cedar oil. This colors bacteria in the vacuoles and the medium as well as the peripheral granules.

OBSERVATIONS

Cytoplasmic Constituents of the Vegetative Individual

A study of the cytoplasm of the vegetative phase of the life cycle of *Uroleptus mobilis* both in life and under the proper methods of fixation reveals the presence of

(*A*) *Gastric vacuoles* which may contain different types of food in various stages of digestion,

(*B*) *Excretion crystals* which may be present within food vacuoles as well as free in the general cytoplasm,

(*C*) *Several types of granules*, as follows:

1. The first type is evident in the form of round granules situated in rows just under the periplast on the ectoplasm and found to stain intra-vitam with neutral red. They are always present after fixation with osmic acid without acetic or alcohols. The initial use of an alcoholic fixative destroys them and turpentine will remove them unless over osmicated. At no time is division evident, and under certain circumstances they may be eliminated. These granules we will call *peripheral granules*.

2. A second type is found in the form of ovoid or elongate granules more or less regularly distributed in the ectoplasm, and visible, though more sparsely scattered in the endoplasm. Their shape changes and evidence of division is abundant. They stain intra-vitam with Janus Green B and are best demonstrated after Benda or other osmic fixatives. The application of turpentine as a bleaching agent after osmic fixatives does not injure them. This description corresponds to that of *mitochondria* found both in Protozoa and Metazoa.

3. A third type of granules may be seen under the proper methods of fixation, at times as flexed dumb-bells, at others as circles or semicircles of granules, distributed irregularly in

the endoplasm and less frequently in the ectoplasm. This flexed dumb-bell appearance makes them appear almost semicircular at times, as though they were part of a circle, half of which had disappeared. They do not conform altogether to the description of mitochondria, as they tend to blacken rather than to stain and are seen with greatest distinctness when the Kopsch fixative as modified by Bowen and the Nassanov technique are used, and are unaffected by turpentine. They appear to divide or fragment into two or more pieces. From these reactions I provisionally interpret them as homologous with the *Golgi apparatus* of Sporozoa and of metazoan cells.

(*D*) *Vacuoles*

Various *vacuoles* of a temporary or permanent nature are present which exhibit a lipoid membrane about their rims after treatment with osmic fixatives. Among these may be listed *food-vacuoles*, a large *vacuole* which is frequently found at the posterior of the organism, and a *contractile vacuole*. Of the three, the last is the only permanent structure. Though, as Nassanov believes, the tendency of the rim to blacken after the application of the Golgi fixatives may possibly place it in the category of a Golgi apparatus, in this study, however, the *contractile vacuole* will be treated as a structure separate from the Golgi-like elements noted above.

(*E*) *Basal Bodies and a Neuro-Motor System*

A system of minute granules are evident in the ectoplasm, and internal to those of the periphery. They are found at the bases of cilia, membranelles and cirri and are connected with each other by a series of fibrils. These elements respond to the technique used for the differentiation of a *Neuro-Motor* system in ciliates. Though alcoholic as well as osmic fixatives make them somewhat evident, the addition of acetic acid sharpens their outlines.

GASTRIC VACUOLES AND THEIR CONTENTS

Up to the present the type of food used by *Uroleptus* was unknown and food vacuoles had not been described. This led Calkins to question whether the peristome really functioned

for the intake of solid food and inclined him to the opinion that the animal might be saprophytic, feeding on the dissolved proteins of the hay-flour infusion alone.

As has been stated, a richer medium than that employed by Calkins was used omitting the dilution of the infusion by equal amounts of spring water. The added concentration causes greater growth and makes evident numerous large *vacuoles*. They are more frequently located in the centre or posterior of the animal and vary in form and frequency, some being somewhat spindle shaped, others round. Their size is often so spectacular that it was hard to believe they could be *food vacuoles*. A study of the peristomial region, however, reveals the presence of a delicate elongate gullet extending well into the cytoplasm and capable of some distention at its base, where, in a few instances, large *vacuoles* in process of formation have been observed. The length of the gullet can readily account for the elongate or spindle shaped appearance of some of the *vacuoles*.

The contents of the *vacuoles* seem to vary. Staining revealed rod-like structures in the spindle-shaped *vacuoles* and frequently shorter rods in the round *vacuoles;* others contained irregularly shaped material or fluid alone.

A study of the medium was made when twenty-four hours old and again after standing for a week or more. The new medium contained several types of bacteria which when cultured and stained were all found to be Gram negative. Animals taken from this medium were subjected to the same method of staining. The *vacuoles* within revealed the presence of rod-like bacteria identical with those found in the medium. The medium which had stood for a week or more contained an abundance of yellow zooglea upon which animals appeared to be feeding. Irregular masses of the same color could be seen within the *vacuoles* of living animals. Upon staining both medium and animal the material within the *vacuoles* was found to be identical with the zooglea. Occasional yeast cells have also been identified within *vacuoles*.

Evidence of digestion was determined by the use of intra-vitam-alizarin sulphate, introduced under a cover slip con-

taining animals fed on both new and old media. Some of the *vacuoles* in addition to fluid were clearly shown to contain bacteria not yet digested while others of the older medium contained shapeless masses like zooglea. Evidence of digestion was abundant as shown by alizarin-sulphate color.

As digestion progresses *vacuoles* become smaller until they are no longer evident. Instead, small masses of residual granules may be seen throughout the cytoplasm as is typical of holozoic forms (they are especially evident after osmic fixatives or the application of neutral red).

An anal spot has not been determined with certainty. However, there is some evidence that the organism eliminates solid materials at the posterior end and on the concave side where there is frequently a *vacuole.*

From these observations we are justified in concluding that *Uroleptus* can and does take in solid food material and digests the same as indicated by the color indicator, alizarin. We are also justified in concluding that some *vacuoles* contain bacteria or zooglea or both along with the dissolved proteins of the boiled hay-flour mixture.

PRODUCTS OF EXCRETION

Other granules have been found both within food vacuoles in which digestion has gone on for some time as well as free in the cytoplasm. These granules have a very clear-cut outline and are visible in life. They often have the appearance of flexed dumb-bells and look very much like bacteria. They may also be round or possess a spike-like prong. They are found in all preserved material (with the exception of those which have been fixed with chromic acid mixtures) and appear to be very little affected by fixatives or dyes. When living animals are placed under the crossed nicols of the polariscope these structures are distinguished by their birefringence, all other granules remaining obscure. This would place them in the category of the "Corpuscles Biréfringents" of Maupas later called "Exkretkörner" by Schewiakoff. Both investigators consider them as *excretory products.* Schewiakoff determined their nature to be calcium phosphate and found

that they tend to congregate about the contractile vacuoles in the case of Paramecium, eventually disappearing in solution in those areas. I have not found that they congregate about the contractile vacuole but they are found throughout the entire cytoplasm and with the greatest frequency in the posterior or middle regions.

GRANULES

Peripheral Granules

Just under the periplast of *Uroleptus*, on the surface of the ectoplasm, a series of ten or twelve rows of *granules* are present though invisible in life unless stained by some intra-vitam color such as neutral red or Bismarck brown.

In order to make clear the real arrangement of some of the *granules* it is necessary to outline the course of the so-called neuromotor apparatus which I have partially determined but the details of which are not yet complete. There are five diagonally longitudinal fibrils in the ectoplasm running the length of the body on the ventral and ventro-lateral surfaces. At intervals basal granules to which cilia are attached appear on the fibrils. Diagonally transverse fibers connect these five rows. Around the peristome fibrils are present connecting the larger basal elements of the membranelles, while through the centre of the peristome still another fibril runs on which basal granules are present at intervals.

Along the above-mentioned five horizontal latero-ventral and ventral rows of fibrils, *peripheral granules* are present above and external to the basal granules of the cilia. Other granules may accompany those which are longitudinally situated and tend to follow the lines of the diagonally transverse fibers.

Other rows of *granules* are present between these latero-ventral and ventral rows as well as on the dorsal surface. They are much less regular in distribution, however. Linear rows of from two to ten *granules* may follow one another at intervals or they may overlap.

Peripheral granules are always found about the peristome. They seem to take up a position between the membranelles

on one side and along the edge of the peristome on the other side while they also may be found along the fibril which extends through the peristomial area. These are usually of a larger size and are strikingly regular in their distribution. At all times these elements are readily distinguished from the more internally placed and usually more minute basal bodies.

Within certain limits these *peripheral elements* vary in size and number. They may be from one-half to one and one-half μ in diameter. They tend to be more frequent as the animal nears division in interdivisional periods.

Chemically they are lipoid in nature as all osmic fixatives preserve them with great distinctness while the initial application of alcohol destroys them immediately. Neutral red stains them intra-vitam which would place them in the group known as metaplastids or "non-living" elements.

Often these *granules* appear to be swollen, especially when osmic acid is used alone or after the application of the Kopsch fixative for Golgi apparatus. At such times it appears that the central core is relatively clear and the rim is blackened. This would indicate that the *granules* are not chemically homogeneous. Under abnormal conditions they may appear with even greater frequency and of larger size. At such times they have been seen to pass through the periplast and to drop off into the medium.

It is impossible to determine with assurance how these *granules* are maintained. It is certain that they do not divide, and that they are lipoid in nature. Their persistence is apparently due to internal factors. Throughout the cytoplasm single grains as well as clumps of *granules* of a lipoid nature may be seen. Frequently, lipoid *granules* have been seen directly under those on the periphery. In studying the living animal after the application of neutral red, red *granules* were seen to come from the interior of the ectoplasm and to attach themselves to the rows of *peripheral granules*. This was seen, however, just before the animal died and may not have been a normal process.

There are two possibilities concerning their function. They may be reserve products which have appeared along the areas

of greatest permeability or where there are elevations or ridges, (along the longitudinal and transverse fibrils of the neuromotor system) or they may be products of an excretory nature. Both the fact that under abnormal conditions they appear with increased size and frequency and have been seen to pass through the periplast and to drop off into the medium, as well as their behavior during encystment (which will be discussed later) would indicate that they are of an excretory nature.

Mitochondria

Throughout the cytoplasm another type of granule may be indistinctly seen *in vivo* without the aid of vital dyes. They appear as constant, homogeneous elements more or less regularly distributed in the ectoplasm, internal to the rows of peripheral granules and are also to be found more sparsely distributed in the endoplasm. Neutral red applied intra-vitam will not affect them while the application of a dilute solution of Janus Green B will color them sharply.

Not only do they tend to be more numerous than the granules of the periphery but they differ from them also in appearance in that they are ovoid and a trifle longer than the former. This difference is accentuated as the animal approaches the division period.

They are best demonstrated by the Benda-Bensley fixatives and dyes and can also be well shown after Schaudinn's. If this fixative be followed by post-osmication using 2% osmic alone or mixed with equal parts of 5% potassium bichromate staining afterwards with iron haematoxylin, the granules appear with great distinctness. Neither alcohol nor turpentine will destroy them but acetic acid if applied in any appreciable amounts will make their demonstration impossible. The reaction of these granules places them in the category of *mitochondria*.

I have been unable to determine any function which these granules may possess in the vegetative individual. *Mitochondria* of *Uroleptus mobilis* have furnished no data for the theory that they manufacture or are metamorphosed into deutoplasmic granules or other cell inclusions. Some evidence

of swelling may be seen in abnormal individuals. There is no evidence that they give rise to the granules of the periphery beyond the fact that the latter lie just exterior to them.

Mitochondria and Bacteria

Since there has been so much controversy recently concerning the nature of *mitochondria* and their possible confusion with *bacteria*, a simple effort was made to determine whether the structures herein termed *mitochondria* would take the Giemsa and Gram bacterial stains and whether any *bacteria* could be found in the cytoplasm. This was done when an examination of the bacterial content of the food vacuoles was being made. The technique employed was as follows:

Five *Uroleptus* were washed one at a time, and each subjected to the following process: With a sterile pipette an animal was placed in a drop of sterile medium—this process was repeated ten times, a new sterile pipette and drop being used for each operation. At the end of this washing, the purpose of which was to free the animal from any *bacteria* which might be adhering to its surface, each animal was smeared upon an agar-medium mixture in a marked-off petri dish, under the proper sterile conditions, and put aside for a few days. The results were as follows: one smear showed nothing; which indicated that the animal was washed sterile, and that had there been *bacteria* present they were unable to exist when that cytoplasm died.

The smear of the four other individuals showed bacterial growth. It appeared that these *bacteria* had the same method of growth as those found in the medium, and when stained were found to be Gram negative, and gave the same appearance as those in the medium. From these facts I concluded that if the four animals had really been washed sterile, and the *bacteria* came then from within, they must be of a type similar to those found in the medium.

Upon staining the animals themselves it was found that the vacuoles alone contained *bacteria*, all evidence of them in the cytoplasm being absent. The only structures in the cytoplasm responding to these stains were the peripheral granules, a

few fatty granules in the endoplasm and in the food vacuoles. No trace of *mitochondria* or *bacteria-like* organisms could be seen. I concluded then that the four animals just mentioned had not been washed sterile, but that some of the *bacteria* of the medium had remained on them, and that if there are *bacteria* in the cytoplasm they do not stain with the Giemsa or Gram technique. Further, the structures termed *mitochondria* are likewise unaffected and to not react to this bacterial technique.

Golgi Elements

There remains one further type of granule in the cytoplasm of the vegetative individual not yet examined and which is not visible in life either with or without the vital stains. They are best seen after fixation with osmic-acid mixtures and because of their special tendency to blacken rather than to stain. I provisionally call them *Golgi elements*. These structures may appear in more than one form. Their general tendency is circular. They may appear as a complete rim of a circle or the circle may be broken up into granules which may be even smaller than mitochondria or they may appear as the rim of a semicircle which at times may have a constriction giving the appearance of a flexed dumb-bell. This last type is the usual one. The *Golgi elements* are found in great abundance throughout the cytoplasm but with greatest frequency in the endoplasm. There is no regularity to their distribution in the vegetative form.

It is sometimes difficult to differentiate the flexed form from the mitochondria, when the mitochondria have not stained well and the *Golgi-like bodies* are not well fixed and have not blackened. At such times the latter elements may be only partially visible and the difference in size between the two types of lipoid structures is diminished. The Golgi structures may be clearly shown, however, by the use of either potassium bichromate-osmic and chromic acid fixatives followed by post-osmication (as advocated by Nassanov), or Bowen's modification of Mann's sublimate osmic fixative followed by osmic acid for several days. In these cases the *Golgi elements* are clearly seen while the mitochondria are not so obvious if

visible at all. Difficulty may sometimes arise in distinguishing the excretory crystals which are often of a flexed form from the *Golgi elements* when the blackening has not been perfect.

Vacuoles

The *contractile vacuole* after the application of osmic-acid mixtures reveals a delicate membrane about its rim.

Another *vacuole* of considerable size is almost always present at the posterior of the form which also displays evidence of a delicate membrane wall. It may assist in the elimination of residual material.

Food vacuoles when present also clearly indicate the presence of a lipoid rim after the use of osmic fixatives.

Division Period of Uroleptus Mobilis

Dividing individuals may be seen in great numbers twenty-four hours or more after placing a mass of *Uroleptus* in a fresh medium. The first external evidence of division is manifested by a shortening of the body and a thickening first of the anterior end and later of its entire length, a new peristome for the daughter individual appearing just posterior to the old one. This shifts posteriorally after which a constriction appears just anterior to it, and the animal elongates and divides into two smaller individuals.

All of the cytoplasmic structures visible in the vegetative individual are present at the time of division. In addition *granules* are evident with osmic fixatives which seem to have a nuclear origin. The macronuclei in preparation for division throw off substances prior to fusing into a single mass all of which are absorbed by the protoplasm.

Peripheral Granules

Upon staining with the proper fixatives *peripheral granules* are seen in great numbers lying closer together than usual. This is not totally accounted for by shortening of the body, as the diagonally transverse rows of granules adhering to the five longitudinal rows are more numerous than before. The *granules* often appear to be somewhat larger than those of the vegetative individual though size variations are always evident.

An interesting evidence of the appearance of *granules* in a special area is seen in connection with the formation of the new peristome for the daughter individual. At the time of this differentiation and the formation of the membranelles with their basal elements and fibrils, a regular row of *granules* appears, a *granule* being evident between the membranelles and external to the neuromotor apparatus. The internal granules of a lipoid nature noted above which are seen in added abundance just before the animal shows any external evidence of division, seem quite similar to those on the exterior and may very possibly give rise to them. At no time is the slightest evidence of division seen.

Food Vacuoles and Excretion Crystals

It is common to find a large number of *food vacuoles* closely packed with bacteria or other substances throughout the cytoplasm at this time. *Birefringent crystals* are also numerous both in vacuoles and throughout the cytoplasm.

Mitochondria during Division of Uroleptus

When the micronuclei become spindle shaped which is prior to the fusion of the various macronuclei into a single mass, the *mitochondria* have become more elongate. Some dividing *mitochondria* may be seen from this time on throughout the whole division period, including the reconstruction of the daughter individuals. I have never seen them dividing *in vivo* but preparations furnish substantial evidence that they do divide by transverse constriction, which, though frequently clear cut at times may be drawn out into "biscuit" formation. Their number seems constant and there is no evidence that they are perpetuated by the nucleus or that they arise *de novo* from the cytoplasm in divisional periods of the active organism.

Golgi Elements during Division

The crescent-shaped or flexed *Golgi elements* tend to cluster about the nucleus during the division period though they may also be seen to some extent in the general cytoplasm. This is particularly apparent in the young daughter individual. These

elements may divide or fragment into more than two pieces which may or may not be of equal size.

Contractile Vacuole and Neuro-motor Elements

Though a *contractile vacuole* as well as *neuro-motor* elements (at least of the anterior) are formed anew for the new individual the details of their formation have not been determined.

ENCYSTMENT

The only reference in the literature concerning encystment of *Uroleptus mobilis* has been made by Calkins ('19), to the effect that reorganization takes place within the cyst which involves the fragmentation of the macronuclei and their subsequent absorption in the protoplasm, while as a result of the process a renewal of vitality or rejuvenescence is effected. S. C. Dellinger, a student of Prof. Calkins, is working out the finer details of nuclear reorganization. Pending the publication of his results, however, a few observations have been made concerning the grosser nuclear changes, along with the study of the behavior of the cytoplasmic constituents, to determine any possible nucleo-cytoplasmic interrelationships.

General External Appearance

As the animal prepares for encystment it becomes much shorter and broader, swelling in the centre until it is almost a complete sphere, with the exception of the peristome which still protrudes somewhat. After circling about for an hour or more, a delicate cyst wall appears within which the active cilia can be seen on the still moving animal which has now become entirely spherical. When the organism becomes quiet the cyst wall is more evident. The contractile vacuole can be seen throughout this period and continues to function slowly. In two or three days the cytoplasm shrinks from the cyst wall and in its centre an irregular roundish yellow-brown mass appears, visible in life. The organism remains in this condition until changed to a new medium. All evidence of motile organelles as well as the contractile vacuole has disappeared.

After a period ranging from one to six days in a fresh medium, motion begins within the cyst wall, and a new animal

of a totally different appearance from that which encysted is evident. Instead of the round short bodied form, we now have a slender, snake-like animal sufficiently long to coil twice within the cyst wall. It twists about violently and in so doing brings into view an irregular mass of yellow-brown material lying outside of the body, between it and the cyst wall. As the animal moves it bumps this mass about. After continued activity the cyst wall is finally ruptured and the animal emerges, a completely formed, elongate *Uroleptus* whose protoplasm is relatively clear, while the discarded *yellow-brown mass* remains within the cyst.

There seem to be variations in the number of animals that may emerge from a cyst. Though but one single form is usual I have nevertheless seen two animals whirling about completely separated from each other in a number of instances. This phenomenon seems to be fairly common. At another time I observed what appeared to be an incompletely divided animal as it broke through the cyst wall.

Origin of the Voided Mass

A study of the stained cysts reveals the following facts. As encystment begins from eight to ten macronuclei are present and the usual four to six micronuclei which at this time have a somewhat elongate appearance, as though preparing for division. The contractile vacuole is always evident, relatively large, and with osmic fixatives clearly shows a membrane wall. A large amount of chromatin is eliminated into the cytoplasm from the macronuclei which gives the appearance of fragmentation. In reality there are eight or more large masses and many smaller ones. Later the larger remains of the macronuclei converge into one mass, which is more or less homogeneous in appearance while "ghosts" of the smaller fragments may be seen throughout the cytoplasm. (After osmic fixatives these frequently appear as clumps of granules.)

During the fragmentation of the macronuclei and the assemblage of the larger fragments into one mass, the micronuclei are active. The details of the micronuclear divisions have not been closely followed. It is evident that some of these

disintegrate while those which persist give rise to the new micro- and macronuclei. In this state the cyst rests until changed to a new medium. Thereupon the new macronucleus enlarges and the micronuclei keep active (*i. e.*, they have an elongate spindle form). During the enlargement of the macronucleus the old macronuclear fragments which had assembled into a mass and were then deeply staining now begin to lose their staining powers and become largely vacuolated, while at times within the mass particles appear which look like excretion substances.

As development of the new macronucleus goes on the old fragments become more and more amorphous and when the new animal is finally formed this mass is usually completely extruded. (Occasionally some of this mass may be retained in the body of the excysting form.) The old macronuclear fragments must, therefore, be at least a part of the extruded substance seen in the living condition and which we have called the "*yellow-brown mass.*"

In encystment, then, we have a repetition of the situation as found in conjugation, *i. e.*, the fragmentation of the macronucleus causing the extrusion of some chromatic material which fades into the cytoplasm, and the formation of homogeneously staining masses, usually eight or ten, which in the active state of conjugation are absorbed by the cytoplasm while in the cyst they are thrown out after having been clumped into a single irregular mass and having lost their staining qualities.

Cytoplasmic Constituents

Food Vacuoles and Excretory Crystals

Food vacuoles have not been observed in animals which have encysted. They do not seem to do any feeding either just before encystment or conjugation.

A varying number of *excretory crystals*, however, are present throughout the cytoplasm as in the active individual. In every case they have sharp, very distinct outlines and tend to be refringent, though it is usually impossible to demonstrate any birefringence under the polariscope, after fixation. As

usual they are unaffected by stains though they appear as dark objects under certain conditions of illumination.

Peripheral Granules during Encystment

If animals are treated with neutral red just as they begin to encyst it is easy to follow the distribution and course of the *peripheral granules* in life during the initial phases of encystment. As the organism rounds up the *granules* retain a semblance of linear order but are much more closely packed together. When activity stops they tend to become more scattered, remaining, however, on the periphery. Several days later, after the protoplasm has shrunk away from the region of the cyst wall, by the use of the proper fixatives the *granules* may be seen adhering to its inner surface. This may be the periplast of the old organism away from which the cytoplasm has shrunk, and to which some of the granules have adhered. More *granules* are frequently visible on the surface of the shrunken cytoplasm. When reorganization begins *granules* may be seen which are sometimes of a slightly larger size on the surface of the cytoplasm or in the space between the cyst wall and the cytoplasm. These may be new *granules* which are being formed and thrown out by the reorganizing animal or they may be more of the old.

Later when reorganization has been completed and the new animal begins to whirl about within the cyst wall, some *granules* of the old organism may still be seen on the inner surface of the wall, while upon the new animal are clearly defined and relatively regular rows of *granules* which, however, are less numerous than those of the ordinary active form. These probably are new elements.

Encystment therefore, among other things, serves as a means of eliminating *peripheral granules* which would indicate that they are of an excretory nature or at least no longer necessary to the cell.

Mitochondria

Mitochondria are easily demonstrated in the early cyst by the ordinary methods of fixation (Schaudinn). They seem at this time more deeply staining and are somewhat elongate and rather regularly distributed over the ectoplasm and less

regularly in the endoplasm. Later when the cytoplasm has shrunk to about half its original volume within the cyst wall it is difficult to demonstrate clearly the presence of mitochondria or of other extra-nuclear elements for a time. (The proper penetration of fixatives and differentiation of stains are most uncertain at this time.) When, however, the encysted animal has been placed in a new medium and reorganization has begun the protoplasm swells once more and the *mitochondria* can again be seen as elongate rodlets. Evidence of division is not clear though the probabilities are that they do divide during encystment. They condense considerably but can always be made out to some extent even in the most contracted period of encystment. At this time their number seems greatly diminished. The new animal, however, is equipped with the usual supply of *mitochondria.*

Golgi Elements

Golgi elements may be demonstrated by fixation with 2% osmic acid for several hours in the early and late phases of encystment. Care must be exercised not to confuse the frequent, flexed excretory particles with *Golgi elements* which have not blackened totally. The excretory particles can usually be distinguished by their harsh clear cut outlines, yet there are times when it is almost impossible to differentiate them from the Golgi. Crescent-shaped bodies may be found clearly when encystment first begins, with great difficulty when shrinkage has occurred and again clearly when reorganization is being completed. There is evidence that these elements break up into several smaller particles during the later stages of encystment.

Contractile Vacuole

The *contractile vacuole* which when encystment is initiated is large and for a time functional, finally disappears at the time of the protoplasmic shrinkage. It reappears when the reorganization is well under way.

CONJUGATION

During conjugation and the period of reconstruction directly following, the nuclei and the cytoplasm of *Uroleptus*

undergo far reaching changes (Calkins). The protoplasm of the exconjugant is more fluid and the organism must be manipulated with great care to guard against its tendency to burst.

When stained with osmic fixatives, clouds of *lipoid granules* can be made out in the cytoplasm, some of which are in the position occupied by the degenerating old macro- and micronuclei, while others are found about the new and feebly staining macronucleus. These may also be seen in life after the use of neutral red. As reorganization progresses these *granules* are absorbed.

Food Vacuoles and Excretory Crystals

Before, during and directly after conjugation *Uroleptus* does not usually feed. In fact after conjugation the peristome seems to be defective for a time. *Food vacuoles* are then absent though *excretory crystals* may still be found scattered throughout the cytoplasm.

Peripheral Granules

Peripheral granules are found as usual though somewhat less regularly distributed. They frequently display a tendency to swell which is probably an expression of the general protoplasmic activity within. At no time do they take any part in conjugation.

Mitochondria

Mitochondria are as abundant as usual though somewhat less regularly distributed in the ectoplasm. They appear denser and are more readily stained at this time. In the early exconjugant they become more elongate and as the new macronucleus divides and subdivides evidence of division among these elements is apparent.

Golgi Elements

Golgi-like elements may also be found in abundance throughout the cytoplasm. They tend to be more spherical and are conspicuously so in the early exconjugant when the old macronuclei are still evident. These circles may break up into smaller granules.

Just before and during the process of conjugation circular bodies appear at the anterior of the peristome of each conjugant. These bodies may be made up of several small particles (sometimes flexed) or they may be single with a colorless centre. At this time in addition to blackening they may also take a haematoxylin stain after fixation with osmic mixtures. These structures are probably identical with the *Golgi-like structures* noted above in spite of their tendency to stain at this time. The position of these elements would indicate that they take part in the process of conjugation. This, however, is but a conjecture.

Contractile Vacuole

The *contractile vacuole* does not seem to change in any way during the process of conjugation, while the neuromotor apparatus in the anterior of the organism, at least, is not so evident.

DISCUSSION

In the following discussion the cytoplasmic granules found in *Uroleptus mobilis* which include *excretory crystals, peripheral granules, mitochondria* and *Golgi-like structures* will be dealt with in the order cited above.

A. Crystals

Maupas ('83, '85) described the presence of "Corpuscules Biréfringents" in many Protozoa, particularly among those which he claimed were carnivorous. Bütschli and later Schewiakoff ('93) found similar bodies in *Paramecium caudatum* to which Bütschli first gave the name of "Exkretkörner." Both Maupas and Schewiakoff described them as irregularly shaped *crystalline bodies* present singly or in masses both within vacuoles and throughout the cytoplasm, their most conspicuous characteristic being *birefringence* under the crossed nicols of the polariscope.

Maupas ('83) describes them as follows: "Dans les groupes—types trouvés chez *Cryptochilum elegans, Uronema marina* et *Euplotes charon,* la forme dominante et presque exclusive est celle d'un corps oblong renflé plus ou moins à ses deux extremités, étrangle au contraire au milieu et rapellant beau-

coup la forme de Bacteriés. Ils sont doués d'une propriété optique qui permet de les distinguer avec la plus grande sûreté. Ils sont, en effet, éminemment biréfringents, et quand on les observe à la lumière polarisée avec les nicols croisés, ils apparaissent avec un éclat très vif sur le fond obscur. Les autres granulations ne possèdent pas la même propriété et restent obscures."

Their chemical properties both investigators found to be: relatively insoluble in water, alcohol, ether, picric acid; slightly soluble in ammonia and acetic; very soluble in potassium bichromate, sulfuric, hydrochloric and nitric acids, chloride of zinc and chromic acid; apparently not affected by osmic acid.

The *birefringent crystals* of *Uroleptus* seem to be identical in appearance and reaction to those described by Maupas for *Cryptochilum elegans*, *Uronema marina*, and *Euplotes charon*.

Maupas, in the case of *Coleps hirtus* which normally contains crystals, states that at times he has been unable to find them and that their absence is due to a change from a carnivorous to an herbivorous diet (Zooglea).

Schewiakoff ('93) in the case of *Paramecium* found that small *crystals* were present after feeding the animal on hay infusion and bacteria while those of a much larger size could be seen if the medium also contained meat extracts or dissolved egg albumin. These *crystals* he determined are composed of calcium phosphate which is found in considerable quantity in both muscle and egg albumin.

Uroleptus may feed on bacteria in an active state or a zooglea condition, as well as on fluid medium, in any case *crystals* are usually present. It is not imperative that the organism be carnivorous as Maupas states but it would seem that the fluid extracts of the nutrient medium on which bacteria and the animal thrive must have as much to do with the formation of *crystals* as any organism which it may have swallowed.

B. Peripheral Granules

It is difficult to homologize the numerous *granules* described as present upon the surface of various Protozoa since in most

cases their chemical nature, origin and function are matters of conjecture, while their fates are known in but a few instances.

Maupas, ('83) in the case of *Uroleptus roscovianis*, found rows of *granules* on its surface after the application of rose carmine. Wallengren ('93) found large and small *granules* distributed on and just under the surface of *Pleurocoptes hydractiniae* after the use of Bismarck brown. Prowazek ('97) with neutral red found *granules* on the extremities and about the lower part of the peristome in the case of *Paramecium aurelia* and *bursaria* to which he gave the name "Exkretperlen." This was confirmed by Slonimski and Zweibaum ('22) in the case of *Paramecium* caudatum. Prowazek also found bodies of a similar nature in rows on the surface of *Pleuronema crysalis*, *Monas vivipara* and the flagellated phase of *Amoeba radiata* Fauré-Fremiet ('10) in the case of *Mycterothrix tuamotuensis*. describes small vacuoles with a thick refringent periphery in the cytoplasm which are thrown out at the moment of encystment. Yocom ('18) in *Euplotes patella* describes rows of rosettes of *granules* on its dorsal surface, each rosette being made up of eight or ten *granules*. These stain with neutral red and are fixed with osmic acid, other fixatives leaving only a vacuole.

In the case of *Pleurocoptes hydractiniae* the *granules* may appear as minute vacuoles containing a granule. The whole vacuole may be pressed through the periplast and thrown off by the action of the cilia. In the case of *Paramecium aurelia* and *bursaria* as well as *Paramecium caudatum* the *granules* may also be lost in the medium.

The origin of the various *granules* is somewhat indefinite as is the case of *Uroleptus mobilis*. Wallengren believes with Prowazek that they are of an excretory nature. Prowazek considers that they appear sometime after the animals have been richly fed, while Slonimski and Zweibaum consider that they may pick up material in the protoplasm such as neutral red and that new *granules* may be formed until the coloring in the protoplasm is eliminated. Fauré-Fremiet in *Mycterothrix* finds these *granules* to be the direct residue of food vacuoles, eliminated, however, only at the time of cyst formation.

The *granules* of *Uroleptus mobilis* seem to be the only ones thus far studied with various fixatives throughout the life cycle of an organism and the fact that they too may be thrown off during the active phases under certain conditions and that they may be eliminated during encystment is rather strong evidence for the conclusion that they are of an excretory nature.

C. Mitochondria

The presence of *mitochondria* has been determined in at least one member of each of the four groups of the Protozoa. The following investigators have contributed to these findings:

Benda, H. C.
1899
Balantidium entozoon

Fauré-Fremiet, E.
1909
Amoeba gorgonia
Chilomonas paramecium
Noctiluca
Opisthonecta henneguyi
Vorticelli convallaria
Trichodinopsis paradoxa
Tintinnidium inquilinum
Urostyla grandis
Opercularia notonecta
Mycterothrix tuamotuensis
Cochliopodium pellucidum
Paramecium caudatum
Glaucoma piriformis
Spirostomum ambiguum
Trachelius ovum
Opercularia racemosa
Carchesium polypinum
Cyttarrocyclis ehrenbergii
Strobilidium gyrans
1912
Condylostoma patens
Epiclintes ambiguus
Fabrea salina
Strombidium sulcatum
Strombidium stylifer

Viguier and Weber
1912
Haemogregarina sergentium

Arndt, A.
1914
Amoeba chondrophora

Leger and Duboscq
1916
Balantidium elongatum

Poisson, R.
1921
Balantidium (sp.)

Dehorne, A.
1921
Euglena — Paramecium caudatum

Cutler, C. W.
1922
Ditrichomonas

Hirschler
1914
Monocystis ascidiæ
1924
Monocystis agilis — Gregarina polymorpha
Gregarina blattarum — Opalina ranarum
Spirostomum ambiguum

Joyet-Lavergne
1924
Gregarina polymorpha — Nina gracilis
Gregarina cuneata — Steinina ovalis
Adelina dimidiata — Aggregata eberthi

Nassanov
1924
Zoothamnium arbuscula — Vorticella (sp.)
Campanella umbellaria — Epistylis gallea
Paramecium caudatum — Chilomonas paramecium

In most cases observations have been made in connection with investigation of other structures. Hirschler, Joyet-Lavergne and Nassanov describe *mitochondria* in connection with a Golgi apparatus, while Cutler mentions them in connection with the general description of the morphology of a parasitic flagellate. These investigators have contented themselves with statements of the presence, general distribution or staining reactions of the *mitochondria* found. Fauré-Fremiet, on the other hand, has made a far more exhaustive study of these elements. In his monograph ('09) he not only reviews the work of previous investigators who have mentioned granules which were probably *mitochondria* though given other names, but in addition he has contributed substantial evidence of the perpetuation of these elements through

division. (He is the sole investigator to have seen the division of *mitochondria in vivo*.) Further, on the basis of observed morphological changes and differing chemical reactions he attributes to the *mitochondria* some physiological functions (elaborators of deutoplasmic substances).

In the following diagrams I have attempted to indicate the findings of investigators of *mitochondria* in the various Protozoa, taking into account size, distribution both general and special, method of perpetuation if, or when, noted. Statements concerning peculiarities of a chemical or physiological nature in the active or encysted condition have also been added. It will be noted that information in the majority of instances is sparse.

Mitochondria of Sporozoa

Name of Animal	Host	General Appearance and Distribution	Author
Monocystis ascidiae	Ascidian	Small, round, numerous throughout cell	Hirschler
Monocystis agilis	Ascidian	Small, round, numerous throughout cell	Hirschler
Haemogregarina sergentium	Gongylus ocellatus	New small granules at extremities	Viguier & Weber
Gregarina blattarum		Short rods frequent, irregular, curved	Hirschler
Gregarina polymorpha		Short rods frequent, irregular, curved	Hirschler
Gregarina polymorpha	Tenebrio molitor	Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne
Gregarina cuneata	Tenebrio molitor	Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne
Nina gracilis	Scolopendra	Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne
Steinina ovalis	Tenebrio molitor	Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne

Organism	Size and Appearance	Distribution		Ti
		General	Special	
Sarcodina				
Amoeba gorgonia	Small, round	In endoplasm	Not in pseudopodia	
Cochliopodium pellucidum	Small, round	In endoplasm	Not in pseudopodia	
Amoeba chondrophora	Small, round			
Mastigophora				
Chilomonas paramecium	Small, round	Throughout cytoplasm	Sides of gullet	
Cryptomonas	Small, round	Throughout cytoplasm		
Noctiluca	Small, round	Throughout cytoplasm	In tentacle	
Ditrichomonas	Ovoid	Throughout cytoplasm	About axostyle	
Euglena (sp.)	Long chondrioconts	Throughout cytoplasm		
Infusoria				
Opalina ranarum	Rod-like, numerous and curved	Throughout cytoplasm		
Paramecium caudatum	Numerous, ovoid (F. F.) Rod-like (Nassanov)	In ectoplasm	Between trichocysts	
Glaucoma piriformis	Long, chondrioconts, 2–5 microns	In ectoplasm	Perinuclear and extremities	
Trachelius ovum	Ovoid, $1\frac{1}{2}$ micron w. elongate, deeply colored rim in endo	Throughout cytoplasm	None about cont. vac.	
Mycterothrix tuamotuensis	Presence noted			
Carchesium polypinum	Ovoid, $\frac{1}{3}$–1 micron Vacuoles—with internal granules	Throughout cytoplasm; irregular, numerous	In stalk and about reservoir of cont. vac.	
Zoothamnium arbuscula	Very small, numerous	Throughout cytoplasm		
Opisthonecta henneguyi	Roundish, 1 micron	More frequent in ectoplasm		
Vorticella convallaria	Small, refringent, vacuolated (seen in vivo), peripheral	Throughout, many in ectoplasm	In stalk and about reservoir of cont. vac.	
Opercularia notonectae	Numerous, 1 micron	More frequent in ant.	Scant near stalk	
Opercularia racemosa	Very small, less than $\frac{1}{2}$ micron, may be diplosomes, short rods	More frequent in ant.		
Campanella umbellaria	Rod-like, flexed	Irregular, scattered	About cont. vac.	
Epistylis gallea	Numerous	Throughout cytoplasm	About cont. vac.	
Trichodinopsis paradoxa	1 micron	In ectoplasm	Fewer in endo.	
Condylostoma patens	Very numerous	Throughout cytoplasm	Fewer in endo.	
Balantidium (sp.)	Rod-like, chondrioconts	In ectoplasm	About nucleus	
Balantidium elongatum	Chondrioconts, 3 μ or more	In ectoplasm at angles to surf.	Rarely in endo.	
Balantidium entozoon	Ovoid, numerous	In ectoplasm		
Spirostomum ambiguum	Rod-like, curved rodlets	Throughout cytoplasm		
Fabrea salina	Round	Throughout cytoplasm	Between membranelles	
Tintinnidium inquilinum	1 micron, roundish			
Cyttarrocyclis ehrenbergii	Round	Irregular throughout cytoplasm		
Strobilidium gyrans	1 micron, round; rim colors more than centre	Irregular throughout cytoplasm	Masses near periphery	
Strombidium sulcatum	Very numerous	Throughout cytoplasm		
Strombidium stylifer	Very numerous	Throughout cytoplasm		
Epiclintes ambiguus	Numerous, round	Throughout cytoplasm		
Urostyla grandis	Ovoid, 1 micron, homogeneous always	Throughout cytoplasm		
Uroleptus mobilis	Ovoid to rods, 1 to $1\frac{1}{2}$ μ long, normally homogeneous	In ectoplasm, less frequent in endo. Somewhat regular	About cont. vac.	

CHONDRIA sion ppearance	Encystment	Reactions to Fixatives	Physiol. role	Author
		Usual technique Benda, Bensley, etc.		Fauré-Fremiet
	Appearance similar	Usual technique Benda, Bensley, etc.		Fauré-Fremiet
	About nucleus	Usual technique Benda, Bensley, etc.		Arndt
		Usual technique Benda, Bensley, etc.		Fauré-Fremiet
		Usual technique Benda, Bensley, etc.		Fauré-Fremiet
		Usual technique Benda, Bensley, etc.		Fauré-Fremiet
		Usual technique Benda, Bensley, etc.		Cutler
			Possible formers of plastids	Dehorne
		Usual technique Benda, Bensley, etc.		Hirschler
		Osmic used Champy-Kull	Deutoplasmic elements	Dehorne Fauré-Fremiet Nassanov
at nuclear irregular		Need special technique		Fauré-Fremiet
				Fauré-Fremiet
				Fauré-Fremiet
iclear divi- in vivo		Very sensitive, often swell		Fauré-Fremiet
				Nassanov
	Easy to fix, dense, smaller	Need special technique		Fauré-Fremiet
liv. period				Fauré-Fremiet
		Seen with ordinary fixatives		Fauré-Fremiet
of animal; omogeneous		Special methods necessary		Fauré-Fremiet
				Nassanov
				Nassanov
		Seen after ordinary fixatives		Fauré-Fremiet
				Fauré-Fremiet
endent of org. may			Form fibrils	Poisson Leger & Duboscq
liv. Biscuit, omogeneous				Benda Fauré-Fremiet Hirschler
		Stain with neutral red		Fauré-Fremiet Fauré-Fremiet
				Fauré-Fremiet
liv. Biscuit, nogeneous ds ds		Stain after ordinary fixatives		Fauré-Fremiet Fauré-Fremiet Fauré-Fremiet Fauré-Fremiet Fauré-Fremiet
division acteria-like nuclear in cell di- encystment on Biscuit	Denser more readily stained Readily stain Denser	Relatively easy to stain Stain after ordinary fixatives		Lowther

Name of Animal	Host	General Appearance and Distribution	Author
Adelina dimidiata	Scolopendra	Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne
Aggregata eberthi		Chondrioconts throughout cytoplasm made up of granules	Joyet-Lavergne

Zoja brothers described "fucsinofile" bodies in number of Protozoa but they have not been included in the chart as mitochondria. A. Alexeieff (16a, b; 17a, b, c, d, e) has described the presence of mitochondria in numerous Flagellates, Sporozoa and Ciliates, from which he derives all the formed elements of the cell. He considers chromidia synonomous with *mitochondria* which come from the nucleus. These may form *glycoplasts, adipoplasts, amyloplasts, chromoplasts* and all the *kinetic elements; parabasal body, blepharoplasts, centrosomes.* As the evidence for these statements is not conclusive, the forms discussed have not been included in this chart.

Mitochondria in the majority of instances appear to be homogeneously staining, roundish, ovoid or rod-like structures varying from one half to one and one-half microns in size, in a few instances reaching a length of three microns or more.

Though usually distributed throughout the cytoplasm they are more abundant in the ectoplasm. In some cases evidence of special distribution has been recorded. They may be in contact with or close to the reservoir of contractile vacuoles (Fauré-Fremiet), or the vacuoles themselves (Nassanov), or distributed in the stalk of a *Vorticella*, and the tentacle of *Noctiluca* (Fauré-Fremiet). Poisson found in *Balantidium*, Joyet-Lavergne and Hirschler in Sporozoa that they tend to clump about the nucleus at some time in the life history of the organism.

Division of these elements has been described by Fauré-Fremiet and Leger and Duboscq. Division may be accomplished during the period of division of the organism (Fauré-Fremiet), or they may divide at any time (Leger and Duboscq). At this time they are invariably homogeneous and deeply staining. The division figures may take the form of biscuits (dumb-bells) or they may have sharp transverse cleavages which give them the appearance of bacteria.

Instances of vacuolated *mitochondria* have been noted and those possessing a more deeply staining outer rim under both

normal and abnormal conditions. These have also been found *in vivo*. In the case of *Paramecium caudatum* some of whose *mitochondria* are normally vacuolated, granules may be seen either attached to the rim or free in their interior. This Fauré-Fremiet offers as evidence of a physiological activity in which *mitochondria* are elaborating deutoplasmic substances. Under abnormal conditions, *mitochondria* may swell by the use of certain reagents, through contact with water or other undetermined causes. Leger and Duboscq consider that some of the *chondrioconts* of *Balantidium* transform into fibrils of this organism.

Uroleptus mobilis is closely related to the hypotrich, *Urostyla grandis* (Fauré-Fremiet), and although *Uroleptus* is a somewhat smaller organism the *mitochondria* of both forms behave in a strikingly similar manner. Normally they are homogeneous and not difficult to stain, in either the active or encysted conditions. In each, elongation of the ovoid *mitochondria* precedes their division which is frequently accomplished by an abrupt transverse cleavage of the rodlets which gives them a bacteria-like appearance. Division figures may be seen during the entire period of division of the organism, in both cases.

The general presence and behavior of *mitochondria* in the active phases and in the encysted condition of *Uroleptus mobilis* leads me to conclude with Fauré-Fremiet and Hirschler that *mitochondria* are constant self-perpetuating elements of the cell.

D. Golgi Elements

Golgi elements were first described in Protozoa by Hirschler ('14) in the case of the Gregarine, *Monocystis ascidiae*, parasitic in an ascidian. He found that along with numerous, minute, spherical mitochondria there were some larger, less numerous lipoid structures, with either a vesicular or membranous appearance or of half rings (crescents) which respond to the special technical methods for the demonstration of the *Golgi apparatus* in the metazoan cell. Later he confirmed these findings and described the same type of structures in the case of *Monocystis agilis* ('24).

King and Gatenby ('23) have found a *Golgi apparatus* in the coccidian *Adelea*, parasitic in the gut of *Lithobius forficatus*. These structures consist "mostly of very characteristically shaped crescents and beads, known in the case of Metazoa as *dictyosomes*." "They occupy an excentric juxta-nuclear position and spread out in the cell protoplasm as does the Golgi apparatus in many metazoan cells." At times they assume the form of large coarse rings or crescents which may clump into a mass.

Joyet-Lavergne ('24a, b, c) distinguishes two types of "lipoïdifères" structures in the Sporozoa, *Aggregata eberthi*, *Nina gracilis*, *Adelina dimidiata*, *Gregarina polymorpha*, *Steinina ovalis*, parasites in the intestine of Tenebrio molitor and Scolopendra. In the early growth periods these granular types which at first are not sharply differentiated later can be separated into linear granules which become chondrioconts, and thick and shorter rodlets which assume a crescent shape or may become granular and clump into masses. The latter he concludes are *Golgi elements*. They are typically distributed in the cytoplasm unless a centriole be present (*Adelina*) when they tend to clump about it.

Evidence of division has been seen by both King and Gatenby and Joyet-Lavergne. The *Golgi elements* tend to take up a position near the nucleus at the time of nuclear divisions in both the cases of spore and gamete formations and seem to keep pace with nuclear multiplication.

These authors have also described some evidence of anisogamy as shown by differences in frequency of the elements among the conjugants. In the case of *Adelea* (King and Gatenby) the female gametocytes are described as possessing scattered *Golgi elements* while the male gametocytes either do not have any or they may be transformed into small granules which lie on the periphery. The authors are in doubt as to the nature of the latter, but believe they are extruded. *Nina gracilis*, *Gregarina polymorpha*, *Gregarina cuneata* and *Steinina ovalis* (Joyet-Lavergne) also give evidence of differing quantities of *Golgi elements*. In the case of *Gregarina polymorpha* the primite is pictured as scantily supplied

with curved rodlets in the posterior end while the satellite has an abundant supply throughout its cytoplasm.

Nassanov ('24) has offered a new hypothesis for the interpretation of a *Golgi apparatus* in Protozoa. In a study of some Peritricha, Holotricha, and a Flagellate, he arrives at the conclusion that the contractile vacuole of these forms has a permanent membrane of a lipoid nature which responds to the Golgi technique and is in reality the homologue of the *Golgi apparatus* of the Metazoan cell. He finds this membrane to be a spherical structure possessing either a heavy wall (Peritricha and some Holotricha) which collapses when the fluid is extruded, or is a thin walled structure (*Paramecium*) which seems to contract upon elimination of fluids. In the case of *Paramecium caudatum* the "apparatus" is complicated by the addition of canals which also have membranes made visible by the Golgi technique. He compares the round rim in the anterior of the Flagellate, *Chilomonas paramecium*, with a similar appearing structure in the collar cells of a fresh water sponge *Spongilla fluviatilis*, illustrated by Hirschler. Both of these structures he considers as membranes of contractile vacuoles. These ring-like bodies he further compares with the spherical type of *Golgi apparatus* found in the germ cell of *Limneaus stagnalis* (Hirschler). Attention is called to the fact that in the cells of the lower vertebrates as well as in the embryonic cells of the higher forms *Golgi elements* are found scattered throughout the protoplasm in the form of flexed rods, crescents, etc., comparable to those found by Hirschler ('14) in the parasite *Monocystis ascidiae*.

Nassanov ('23) has produced evidence that the function of the *Golgi apparatus* in the Metazoa is secretory, a hypothesis also held by Bowen. He attributes a similar function to the membrane of the contractile vacuole of Protozoa, since a fluid is collected within its wall which it expels. He does not attempt an analysis of the nature of this fluid, though he offers various suggestions as to the role played by the membrane in its collection or formation.

Summing up our findings of various writers on the *Golgi apparatus* of Protozoa; Hirschler, King and Gatenby, Joyet-

Lavergne, state that the structures may be scattered or in aggregates, the elements of which may be single, spherical or crescentic, flexed rods or granules. They may be unequally distributed in the sexual phases in some cases and seem to keep pace with the formation of nuclei in spore and gamete formations. Nassanov on the other hand makes no mention of any scattered type of *Golgi apparatus* in ciliates or the one Flagellate studied but finds the contractile vacuole membrane the only structure responsive to the Golgi technique.

Nassanov's work on the Protozoa is a spectacularly clever piece of research which contains valuable information, especially on two debated points: in the first place proof is given that the contractile vacuole is a permanent organ, secondly that it possesses a membrane. The latter observation has been confirmed by R. Howland ('23) for living *Amoeba* and *Paramecium*, and the former by R. A. Young ('24) for *Paramecium*.

King and Gatenby ('25), although they disagree with Nassanov's conclusions nevertheless confirm his findings of the presence of a lipoid membrane about the contractile vacuole.

The investigation of the cytoplasm of *Uroleplus mobilis* with the special technique necessary for the determination of a *Golgi apparatus* in Metazoa, reveals the presence of an extremely delicate lipoid membrane about the contractile vacuole, and also many scattered elements which may be spherical, hemispherical or flexed rodlets which seem similar to the elements found in the Sporozoa. These findings lead me to the conclusion that the membrane about the contractile vacuole probably has nothing to do with the *Golgi apparatus* of ciliates but is merely a lipoid structure responding to osmic acid much as the membranes of other vacuoles would do. That it is a part of the *Golgi apparatus* which has become specialized, contractile, and definitely localized seems on the whole improbable.

SUMMARY

The study of the cytoplasmic elements of *Uroleptus mobilis* during the entire life cycle reveals the following facts, most of which have become evident after the use of osmic-acid fixatives.

1. A *cytostome* containing two membranes in addition to the membranelles is present and is capable of some distention at its posterior end where *food vacuoles* are formed.

2. *Uroleptus* may take in and digest solid as well as liquid food as seen in *vacuoles* present in richly fed organisms. These are absent during conjugation and encystment. Residual food masses are frequently present in the active phases.

3. *Excretory crystals* similar to those found by Maupas (called "corpuscules birefringents") and Schewiakoff (called "Exkretkörner") which exhibit birefringence under the crossed nicols of the polariscope, are found throughout the life cycle in both active and encysted conditions. Evidence of their elimination is seen during encystment.

4. *Peripheral granules* comparable to those found by Wallengren, Prowazek and Slonimski and Zweibaum and which are called "Exkretperlen" are present in *Uroleptus*. They are situated in rows external to the ectoplasm above the fibrils of the neuro-motor system. They stain with neutral red and are of a lipoid nature since they are destroyed by the initial use of alcohol and by turpentine. They have never been seen to divide, may be thrown off under certain circumstances in the active individual and are eliminated during encystment; new granules appearing upon the excysting organism. Like the "Exkretperlen" of Prowazek they are probably of an excretory nature, having their origin through the general metabolic activities of the cell.

5. *Mitochondria* which correspond to those described by Fauré-Fremiet in the closely related organism *Urostyla grandis* are found more or less regularly distributed in the ectoplasm, less frequently in the endoplasm. Their number seem constant during the active phases of the cycle, becoming denser before division, at conjugation and during encystment. They are normally homogeneous, appearing as ovoid or elongate bodies which take the usual mitochondria stains though they may also be demonstrated after the use of Schaudinn fixative if employed for a short time. They are self perpetuating, dividing at the time of the nuclear activity of the cell. During encystment they become less frequent and some may possibly be formed *de novo* as the animal begins reorganization.

6. Evidence of *bacteria* in the cytoplasm other than that found in food vacuoles is entirely negative as shown by the Gram and Giemsa stains. *Mitochondria* of Uroleptus do not respond to this technique.

7. Scattered *Golgi-like elements* appear irregularly distributed in the endoplasm and less frequently in the ectoplasm. They have a flexed dumb-bell, crescent-shaped or spherical appearance and are present both in the active phases as well as during encystment. They blacken after prolonged treatment with osmic acid and appear to be similar to those elements found in the Sporozoa which Hirschler, Joyet-Lavergne and King and Gatenby have called a *Golgi apparatus*. They seem to be self-perpetuating, fragmenting or dividing during the nuclear activities, at division and conjugation. In the cyst, however, it is not certain that they may not be formed *de novo*.

8. A *contractile vacuole* is situated on the dorsal surface of the organism which possesses a delicate lipoid membrane about its rim. Two canals extending into the anterior and posterior are also present. This vacuole is present throughout the active phases of the organism, a new vacuole appearing in one of the divided animals. During encystment, however, the vacuole which becomes conspicuously large in the early phases and has a more deeply staining rim, disappears completely as encystment progresses. A new vacuole appears in the excysting organism when the other organelles are reforming. This vacuole with its contractile lipoid membrane is especially conspicuous at this time after the application of osmic fixatives.

9. Nuclear eliminations into the cytoplasm.

(*a*) Just prior to division the macronuclei throw off substances into the cytoplasm which are evident with the osmic acid fixatives. These elements are eventually absorbed by the protoplasm.

(*b*) The macronuclei of the exconjugant also throw off masses into the cytoplasm, some of which tend to collect about the new feebly staining macronucleus. Granules may also be seen in the position of the eight remaining masses of the old macronuclei, and degenerating micronuclei. All of these elements are eventually absorbed.

(*c*) During encystment the macronuclei throw off fragments and later clump into one large mass as at division periods. This at first is deeply staining and corresponds to the eight macronuclear remains found after conjugation. Micronuclei divide and eventually form a new macronucleus as well as new micronuclei. Degenerating micronuclei may also be seen in process of absorption. When excystment is initiated the old mass of macronuclear material which no longer stains well, is extruded from the cell along with some excretory crystals mentioned above.

10. Evidence of the formation of any of the cytoplasmic elements from the nucleus has not been established.

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